

Artificial Intelligence assisted microbial diagnosis: A prospective study to evaluate the diagnostic accuracy and turnaround time

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Abstract

Background: Conventional microbial diagnosis is time-consuming and subject to inter-observer variability, which may delay targeted antimicrobial therapy. Artificial intelligence (AI) has emerged as a promising tool to support rapid and standardized interpretation of microbiological data. **Aim:** To evaluate the diagnostic accuracy, turnaround time, and clinical utility of an AI-assisted microbial diagnostic system compared with conventional microscopy and culture-based methods.

Methods: This prospective observational study was conducted in the Department of Microbiology, Mahaveer Hospital, Pune, from January to August 2025. A total of 420 clinical specimens were processed using routine microbiological techniques. AI-assisted interpretation of stained smears was performed independently and compared with expert microbiologist reports as the reference standard. Diagnostic performance metrics, agreement statistics, ROC analysis, and turnaround time were assessed.

Results: The AI-assisted system demonstrated an overall agreement of 92.1% with expert microscopy ($\kappa = 0.87$). Sensitivity and specificity were 91.8% and 93.5%, respectively. ROC analysis showed excellent discriminatory ability with an AUC of 0.93. The mean turnaround time for AI-assisted preliminary reporting was significantly shorter than conventional methods (2.1 vs 38.4 hours; $p < 0.001$).

Conclusion: AI-assisted microbial diagnosis provides rapid and accurate preliminary results and can effectively complement conventional laboratory workflows.

Key words: Artificial intelligence, Clinical microbiology, Diagnostic accuracy, Gram stain, Turnaround time

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Introduction

Conventional microbial diagnosis relies on microscopy, culture, biochemical tests, MALDI-TOF identification and antimicrobial susceptibility testing (AST); although reliable, these workflows are often limited by turnaround time, inter-observer variability and fragmented data streams, which can delay targeted therapy and infection-control decisions [1]. Recent advances in artificial intelligence (AI) especially machine learning and deep learning are enabling automated interpretation of laboratory images (e.g., Gram-stain slides) and integration of high-dimensional data (spectra, genomics, and clinical metadata) to support faster, more standardized, and potentially earlier organism recognition and resistance prediction [1,2].

AI-assisted systems have already demonstrated practical promise in clinical microbiology, such as convolutional neural network-based support for Gram-stain interpretation from positive blood cultures and automated microscopy platforms for parasitology, helping reduce workload and improve reproducibility while maintaining clinically meaningful agreement with expert reads [2, 3]. At the same time, microbiology-focused AI frameworks emphasize rigorous data preparation, deployment-mirrored validation, and responsible interpretation to avoid bias and preserve safety when models move

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from proof-of-concept to routine diagnostics [4]. The aim of the study is to evaluate the diagnostic utility of AI-assisted approaches in microbial diagnosis by assessing accuracy, turnaround time, and clinical decision impact compared with standard laboratory methods [1, 2].

Methods:

This prospective observational diagnostic accuracy study was conducted in the Department of Microbiology, Mahaveer Hospital, Pune, from January 2025 to August 2025. After obtaining approval from the Institutional Ethics Committee, consecutive patients for whom microbiological investigations were requested by the treating clinicians were enrolled. Written informed consent was obtained wherever required as per institutional policy. Both inpatients and outpatients of all age groups were included if a clinically relevant specimen was received in the laboratory with adequate volume and proper labeling. Specimens that were leaking, grossly contaminated, inadequate in quantity, or received after prolonged transport without appropriate conditions were excluded. Demographic and clinical details (age, sex, ward/OPD, provisional diagnosis, prior antibiotic exposure, and specimen type and collection time) were recorded in a structured proforma.

All specimens were processed according to standard laboratory protocol during the study period. Samples included blood cultures, urine, sputum/endotracheal aspirates, pus/wound swabs, body fluids and other clinically indicated specimens. Direct microscopy was performed as applicable (Gram stain for most specimens; wet mount/KOH where indicated). Blood culture bottles flagged positive were subcultured and subjected to Gram staining. Cultures were inoculated on appropriate media (e.g., blood agar, MacConkey agar, chocolate agar or other selective media where required) and incubated under standard conditions. Organisms were identified using conventional colony morphology, Gram reaction and routine biochemical tests and/or automated identification as available in the laboratory. AST was performed using Kirby–Bauer disc diffusion and interpreted as per prevailing standards followed by the department, and minimum inhibitory concentration (MIC) testing was performed when clinically indicated and feasible. The reference standard for organism identification and AST was the routine laboratory report finalized by the microbiologist after complete work-up and quality checks. Internal quality control measures (media sterility checks, control strains for AST, and periodic equipment calibration) were followed throughout.

For the AI component, digital images of relevant slides (primarily Gram-stained smears from direct specimens and positive blood cultures) were captured using a microscope-mounted camera with standardized illumination and magnification settings. The AI model (image-based classifier) was applied to categorize findings such as Gram-positive cocci in clusters/chains, Gram-negative bacilli, mixed flora, yeast-like cells, and “no organisms seen,” and to generate an AI-assisted preliminary interpretation. The AI output was recorded independently and blinded to the final culture identification at the time of capture. Turnaround times were documented for (i) AI-assisted preliminary result time from sample receipt/flag positivity to AI output, and (ii) routine reporting time for microscopy, culture identification, and AST. Outcomes included agreement between AI interpretation and expert microscopy (percent agreement and Cohen’s kappa), sensitivity/specificity of AI for key categories, discordant analysis with adjudication by a second microbiologist, and impact measures such as proportion of cases where AI could have enabled earlier presumptive organism category reporting. Data were entered into Microsoft Excel and analyzed using SPSS; categorical variables were presented as frequencies/percentages, continuous variables as mean $\pm$ SD or median (IQR) as appropriate. Comparisons of turnaround time were assessed using independent t-test or Mann–Whitney U test, and a p-value <0.05 was considered statistically significant.

Results:

A total of 420 clinical specimens were analyzed during the study period, with blood cultures constituting the most common sample type (32.9%), followed by urine (26.7%) and pus or wound swabs (17.6%) (Table 1). The AI-assisted microscopy system demonstrated a high level of concordance with expert microbiologist interpretation across all organism categories. Overall agreement between AI outputs and conventional microscopy was 92.1%, with a Cohen’s kappa value of 0.87, indicating excellent agreement beyond chance (Table 2). The highest concordance was observed for Gram-negative bacilli (94.1%), followed closely by Gram-positive cocci (92.4%). In terms of diagnostic performance, the AI-assisted approach showed a sensitivity of 91.8% and specificity of 93.5% for detection and categorization of microorganisms on stained smears (Table 3). The negative predictive value was particularly high (94.1%), suggesting reliable exclusion of organisms when AI reported no organisms seen. Discordant cases were mainly related to mixed flora and poorly stained smears, which required adjudication by a second microbiologist. A significant reduction in turnaround time was observed with AI-assisted preliminary reporting. The mean time to obtain an AI-generated interpretation was 2.1 ± 0.6 hours, compared to 38.4 ± 6.8 hours for routine microscopy combined with culture-based identification ($p < 0.001$) (Table 4). Receiver Operating Characteristic (ROC) curve analysis further confirmed the robustness of the AI model. The area under the ROC curve (AUC) was 0.93 (95% CI: 0.90–0.96; $p < 0.001$), indicating excellent discriminatory ability of the AI system in differentiating positive from negative microscopy findings when compared with the reference standard (Figure 1). The ROC curve consistently remained well above the diagonal reference line, reflecting a favorable balance between sensitivity and specificity across multiple thresholds.

Discussion:

The present prospective study demonstrated that artificial intelligence (AI)-assisted microbial diagnosis can significantly enhance the accuracy, speed, and standardization of routine microbiological workflows. In our cohort, the AI model showed excellent agreement with expert microbiologist interpretation ($\kappa = 0.87$) and high diagnostic accuracy, findings that are consistent with recent literature highlighting the growing reliability of AI-based image analysis in clinical microbiology [1, 4]. Traditional microscopy and culture-based methods, although considered gold standards, are labor-intensive and subject to inter-observer variability, particularly in high-volume laboratories. AI-assisted systems address these challenges by providing consistent preliminary interpretations,

thereby supporting microbiologists rather than replacing them [5]. The high concordance observed in our study for Gram-negative bacilli and Gram-positive cocci aligns with previous reports where convolutional neural networks achieved strong performance in recognizing common bacterial morphologies on Gram-stained smears [6].

A key strength of this study was the comprehensive evaluation of diagnostic performance metrics, including sensitivity, specificity, predictive values, and ROC analysis. The observed AUC of 0.93 indicates excellent discriminatory ability of the AI system in differentiating positive from negative microscopy findings, reinforcing its robustness across varying thresholds [7]. Similar AUC values have been reported in studies evaluating AI for Gram stain interpretation from positive blood cultures, suggesting that AI models can reliably support early organism categorization in critical clinical scenarios such as sepsis [2]. The high negative predictive value noted in our study is particularly clinically relevant, as it suggests that AI-reported “no organisms seen” results are dependable, potentially reducing unnecessary empirical escalation of antimicrobial therapy [8]. However, discordant cases mainly involving mixed flora and suboptimally stained smears underscore the importance of maintaining expert oversight and quality control in AI deployment.

Another major finding of this study was the substantial reduction in turnaround time achieved with AI-assisted preliminary reporting. The mean reporting time of just over two hours represents a marked improvement compared with conventional workflows, which often require more than 24–48 hours for organism identification and susceptibility results [9]. Rapid preliminary information is critical in acute care settings, where early initiation of appropriate antimicrobial therapy has been shown to improve patient outcomes and reduce mortality [10]. By providing early organism category information, AI-assisted microscopy can complement antimicrobial stewardship efforts, enabling more rational initial antibiotic selection while awaiting definitive culture results [11]. From a laboratory management perspective, such time savings may also translate into improved workflow efficiency, reduced reporting backlogs, and optimized utilization of skilled microbiology personnel.

Despite these advantages, several challenges and limitations must be considered when interpreting our findings. AI models are highly dependent on the quality and representativeness of the training data, and performance may vary across institutions due to differences in staining techniques, microscope optics, and patient populations [5, 12]. In our study, misclassifications were more frequent in polymicrobial samples and poorly stained smears, highlighting the need for standardized pre-analytical

and analytical procedures. Furthermore, most current AI applications in microbiology focus on image-based tasks, whereas integration with other data streams such as MALDI-TOF spectra, genomic data, and electronic health records remains an evolving area [13]. Ethical considerations, including data privacy, algorithm transparency, and medicolegal responsibility for AI-assisted decisions, also warrant careful attention before widespread clinical adoption [12].

Future research should focus on multicentric validation studies, inclusion of diverse specimen types, and real-world impact assessments on clinical outcomes such as length of hospital stay, antibiotic consumption, and mortality. The integration of AI-assisted microscopy with molecular diagnostics and automated susceptibility prediction represents a promising direction for creating comprehensive decision-support systems in clinical microbiology [1, 13]. In conclusion, our findings support the role of AI as a valuable adjunct to conventional microbiological diagnostics, capable of delivering rapid, accurate, and reproducible preliminary results while preserving the central role of the clinical microbiologist in interpretation and patient care.

Conclusion: This prospective study demonstrated that artificial intelligence–assisted microbial diagnosis is a reliable and efficient adjunct to conventional microbiological methods. The AI system showed excellent agreement with expert microscopy, high diagnostic accuracy, and strong discriminatory ability as reflected by a high AUC on ROC analysis. Importantly, AI-assisted preliminary reporting significantly reduced turnaround time, enabling earlier presumptive organism identification. While challenges such as mixed flora interpretation and dependence on slide quality remain, AI has clear potential to support timely clinical decision-making and antimicrobial stewardship. Integration of AI into routine workflows, with continued expert oversight, can enhance laboratory efficiency and patient care.

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Table 1: Distribution of clinical specimens included in the study

Specimen type	Number (%)
Blood cultures	138 (32.9)
Urine samples	112 (26.7)
Pus / wound swabs	74 (17.6)
Respiratory specimens	62 (14.8)

Body fluids (CSF, pleural)	34 (8.0)
Total	420 (100)

Table 2: Agreement between AI-assisted interpretation and expert microscopy

Microscopy category	Agreement (%)	Kappa value
Gram-positive cocci	92.4	0.86
Gram-negative bacilli	94.1	0.89
Yeast-like cells	90.3	0.82
Mixed flora / no organisms	87.6	0.78
Overall agreement	92.1	0.87

Table 3: Diagnostic performance of AI-assisted microscopy

Parameter	Value (%)
Sensitivity	91.8
Specificity	93.5
PPV	90.9
NPV	94.1
Accuracy	92.4

Table 4: Comparison of turnaround time (TAT) between AI-assisted and routine methods

Method	Mean TAT (hours)	p value
AI-assisted preliminary report	2.1 ± 0.6	<0.001
Routine microscopy + culture	38.4 ± 6.8	

Figure 1: ROC curve demonstrating the diagnostic accuracy of the AI-assisted microbial diagnostic system

